3F11

Biochemical Indicators of Heavy Metal Contaminants in Big Creek, Iron County, Missouri

Project Completion Report to the U.S. Fish and Wildlife Service
Ecological Services Field Office
Columbia, MO

Christopher J. Schmitt
U.S. Geological Survey, Midwest Science Center[a]
4200 New Haven Rd., Columbia, MO 65203

and

Colleen A. Caldwell[b]
U.S. Geological Survey, Upper Mississippi Science Center[a]
2630 Fanta Reed Rd., P.O. Box 818
La Crosse, WI 54620-0818

April 14, 1997

Background and Justification

Southeastern Missouri has been mined for lead and other metals for more than 200 years. During this time, much of the beneficiated ore was transported to Herculaneum, MO for smelting, and the refined lead was subsequently shipped via the Mississippi River. Results of a completed study funded by U.S. Fish and Wildlife Service (FWS) Region 3 (89-3-052; Schmitt et al. 1992, 1993) revealed the presence of anomalously high concentrations of cadmium, lead, and zinc in fish from Big Creek, in southeastern Missouri. Big Creek, which flows through Sam A. Baker State Park *en route* to Lake Wappapello, is classified as an Outstanding State Resource Water by the Missouri Department of Natural Resources (MDNR), which means that it is one of 15 streams statewide that must meet strict water quality criteria (MDNR 1992). The creek receives, via a small branch (Strother Creek), the NPDES-permitted discharge of metals from the American Smelting and Refining Co. (ASARCO) smelter in Glover, as well as intermittent leachates from an abandoned lead mine located near Annapolis (USGS 1967). In June, 1992, the rupture of a dam

---

[a] Formerly National Biological Survey / Service

[b] Present address: U.S. Geological Survey, New Mexico Cooperative Fish and Wildlife Research Unit, New Mexico State University, P.O. Box 30003, Department 4901, Las Cruces, NM 88023

constructed of rhyolite tailings at a roofing materials plant located in Annapolis caused a massive fish kill in Big Creek. It is not known whether the roofing materials plant also constitutes a non-point source of metals to the creek; the plant has no permitted discharge. Schmitt et al. (1992, 1993) reported carcass- and blood-cadmium concentrations in fish from Big Creek that were higher by 10-fold than reference sites, and which were 5-fold higher than those in fish from even the most heavily contaminated sites in Missouri's Old Lead Belt and Tri-States Mining District. Lead and zinc concentrations were also elevated, and the activity of the enzyme δ-aminolevulinic acid dehydratase (ALA-D) was lower in the blood of Big Creek fish than at reference sites. ALA-D is an enzyme involved in the synthesis of hemoglobin; it is a sensitive and specific bioindicator of environmental lead exposure (Hodson et al. 1977; Dwyer et al. 1988; Schmitt et al. 1984). The site sampled in the 1989 study (Schmitt et al. 1992, 1993) was downstream of the smelter discharge, the rhyolite tailings spill, and the intermittent stream draining the abandoned lead mine.

Objectives: This study was designed and conducted to better characterize the nature and extent of the elevated metals detected fish from Big Creek by the earlier study; and to define the source or sources of metals to Big Creek.

METHODS OF STUDY

A total of 22 northern hogsuckers (*Hypentelium nigricans*) were collected by electrofishing from four sites on Big Creek, in Iron Co., Mo. (Fig. 1, Table 1) on August 30 and 31, 1993. Upon collection, the fish were stunned with a blow to the head, and blood samples were obtained via caudal veinipuncture with a heparinized 5-cc syringe and 20-ga needle. One aliquot of blood was dispensed into a labeled Cryovial® and immediately frozen in liquid nitrogen for subsequent analysis of ALA-D activity and hemoglobin (Hb) concentration. These samples were stored frozen at -80° C until analyzed. The remainder of the blood was dispensed into a pre-labeled, acid-cleaned, borosilicate glass tube; chilled (0° C) immediately; then frozen (-20° C) upon return to the laboratory (ca. 24 h). The fish were weighed and measured, then dissected and identified to gender. The carcasses were individually wrapped in foil and also chilled (0° C) until returned to the laboratory, where they too were frozen (-20° C). Budgetary constraints precluded the complete analysis of all 22 blood and carcass samples for elemental contaminants. A subset of blood and carcass samples (Table 1) was selected and shipped frozen to the analytical laboratory for analysis of percent moisture and concentrations of aluminum (Al), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb) by inductively coupled plasma emission spectroscopy (ICPES), with pre-concentration. The analytical laboratory reported dry-weight metals concentrations, which were converted via the moisture content value to wet-weight concentrations for statistical analyses and comparisons with previous investigations. Detection limits were estimated independently for each metal in each sample, and are reported for censored values in Appendix Table A1. Details of all laboratory procedures were presented by Schmitt et al. (1992, 1993). Concentrations of metals and other inorganic constituents were also determined in grab-samples of water collected during the sampling period by the U.S. Environmental Protection Agency, Region VII (EPA) using ICPES.

ALA-D activity was measured as the amount of enzyme-catalyzed product, PBG (porphobilinogen, a hemoglobin precursor), per milligram of blood per hour. Because both ALA-D and Hb occur in erythrocytes, ALA-D activity is typically expressed per unit concentration of Hb (Schmitt et al. 1984). Concentrations of metals in blood and carcass were log-transformed for statistical analysis. To facilitate this and other statistical computations, a value of one-half the detection limit was substituted for censored values. Data were analyzed by one-way analysis of variance using an α-level of 0.05.

RESULTS

The size and weight of the Northern hogsuckers collected from Big Creek generally decreased with distance upstream; specimens from Site 1 (below Annapolis) were generally largest and heaviest, and those from Site 4 (near Hogan) were generally smallest and lightest (Table 2). Among the metals analyzed by ICPES, concentrations of Be in carcass and of Cr in both blood and carcass were below detection levels in all or most samples (Appendix Table A1); however, Be was detected in blood from all fish collected at Sites 3 and 4, but in none from Sites 1 and 2 (Appendix Table A1). For measurable metals in fish carcasses, only concentrations of Al, Cd, Ni, and Pb differed among sites. Concentrations of Al were significantly ($P < 0.05$, Fischer's protected LSD) greater at Sites 2 (above Annapolis) and 4 (Hogan) than at Sites 1 (below Annapolis) and 3 (Glover--Table 2). Concentrations of Ni were significantly greater at Site 4 than at all others; conversely, the mean concentration of Cd was significantly lower at Site 4 than at all others (Table 2). Mean Pb concentrations were significantly greater at Sites 1 and 3 than at Sites 2 and 4 (Table 2). Among metals in blood, only mean concentrations of Cd and Pb differed significantly among sites (Table 3). For Cd, there was an obvious gradient of increasing concentrations from upstream to downstream; concentrations were lowest at Site 4 (Hogan) and greatest at Site 1 (below Annapolis--Table 3). For Pb, concentrations were significantly greater at Sites 1 (below Annapolis) and 3 (at Glover) than at Sites 2 and 4 (Table 3). Contemporaneous data collected by EPA-Region VII also showed elevated concentrations of Pb and Cd, as well as of Zn, at all Big Creek sites downstream of the smelter discharge (J. Pitt, personal communication).

Mean ALA-D activity and ALA-D activity adjusted for Hb (ALA-D / Hb), but not Hb concentrations, differed significantly among sites (Table 3). As expected, ALA-D activity was greater at the sites with lowest blood and carcass Pb concentrations (Sites 2 and 4) than at the sites with elevated Pb (Sites 1 and 3--Table 3, Fig. 2). ALA-D activity was well-described by log-linear relations with blood- and carcass-lead concentrations, with the samples from each site grouped together (Fig. 2).

DISCUSSION AND CONCLUSIONS

The relationship between blood-Pb and ALA-D activity (Fig. 2) is typical of what has been observed in previous Missouri studies (Schmitt et al. 1984, 1993; Dwyer et al. 1988). These and other studies of fishes, as well as investigations of warm-blooded vertebrates, have noted an ameliorative effect of zinc (Zn), probably because of this element's involvement as a cofactor in the activation of the enzyme (Finelli 1977). Budgetary constraints precluded the analysis of Zn in blood or carcass samples in this study; however, both were relatively high at Site 1 in 1989 (Schmitt et al. 1993). The contemporaneous data collected by EPA-Region VII also showed elevated Zn in the waters of Big Creek, and the ASARCO smelter discharges this element, along with others, in its effluent (J. Pitt, personal communication). Consequently, some of the lack-of-fit evident in Fig. 2 is probably a consequence of varying, but unknown, concentrations of blood-Zn in the 1993 fish.

The mean 1993 carcass-Pb concentration of 2.21 μg/g at Site 1 (below Annapolis; Table 2) is lower by about 50% than the mean concentration of 4.57 μg/g reported for that site in 1989 (Schmitt et al. 1993); however, the mean blood-Pb concentration of 1.04 mg/L (Table 3) represents a 68% increase over the 1989 mean of 0.62 mg/L. Both values are about half those reported for suckers from the most heavily contaminated reaches of the Big River, in the Old Lead Belt, in 1989, but exceed those reported for Center Creek, a tailings-contaminated stream in the Tri-State Mining District of southwestern Missouri (Schmitt et al. 1993). In human medicine, blood-Pb levels of 1-1.5 mg/L are considered a cause of concern (Mushack et al. 1989), and avian wildlife is considered Pb-poisoned at blood-Pb levels of 0.2-0.6 mg/L (Anderson et al. 1985). There are no comparable values for fishes; however, avian wildlife is considered Pb-poisoned when ALA-D activity is inhibited by 50% relative to control or reference animals (Dieter 1979), as are some fishes (Hodson et al. 1984). ALA-D activity was depressed by about 50% at Site 1 (below Annapolis) relative to Site 4 (Hogan--Table 3).

Mean 1993 carcass-Cd (1.32-1.82 μg/g) and blood-Cd (0.18-0.44 mg/L) concentrations at Sites 2-4 also exceeded those found at Site 1 in 1993 (carcass-Cd 1.18 μg/g; blood-Cd 0.134 mg/L--Schmitt et al. 1993), the values which prompted this study. The mean 1993 carcass-Cd concentrations at Sites 2-4 also exceed by 6- to 8-fold the maximum carcass-Cd value of 0.22 μg/g wet-weight reported by the National Contaminant Biomonitoring Program (NCBP) in 1984 (Schmitt and Brumbaugh 1990). Collectively, these findings indicate that Pb and Cd are discharged to Big Creek; are available to and accumulated by fishes; and that concentrations are increasing. Two sources--i.e., the smelter and the Annapolis mine--are indicated by the bimodal distributions of blood-Pb, carcass-Pb, and ALA-D activity (Tables 2-3); the gradient in Cd concentrations (Tables 2-3); and the clustering of the samples from each site evident in Fig. 2. The high and rising concentrations of metals in fish from Big Creek warrant continued monitoring.

ACKNOWLEDGMENTS

This study was jointly funded by the National Biological Survey / Service (now the Biological Resources Division of the U.S. Geological Survey) as part of research to validate the use of biomarkers in the Biomonitoring of Environmental Status and Trends (BEST) program; and the U.S. Fish and Wildlife Service, Region 3, through its Columbia, Missouri Ecological Services Field Office. B. Mueller, T. Nash, and B. Poulton assisted in the field. J. Pitt of EPA-Region VII collected and provided for the analysis of water samples as part of this study.

REFERENCES

Anderson, W.L., and S.P. Havera. 1985. Blood lead, porphyrin, and ingested shot for detecting poisoning in waterfowl. *Wildl. Soc. Bull.* 13:26-31.

Dieter, MP. 1979. Blood δ-aminlevulinic acid dehydratase (ALA-D) to monitor lead contamination in canvasback ducks (*Aythya valisneria*). Pp. 177-191 *in* Animals as monitors of environmental pollution. National Academy Press, Washington, D.C.

Dwyer, F.J., C.J. Schmitt, S.E. Finger, and P.M. Mehrle. 1988. Biochemical changes in longear sunfish, *Lepomis megalotis*, associated with lead, cadmium and zinc from mine tailings. *J. Fish Biol.* 33:307-317.

Finelli, V. 1977. Lead, zinc, and δ-amino levulinate dehydratas. Pp. 351-364 *in* S.D. Lee and B. Peirano, eds. Biochemical effects of environmental pollutants. Ann Arbor Science, Ann Arbor, MI.

Hodson, P.V., B.R. Blunt, D.J. Spry, and K. Austen. 1977. Evaluation of erythrocyte δ-amino levulinc acid dehydratase activity as a short-term indicator in fish of a harmful exposure to lead. *J. Fish. Res. Bd. Can.* 25:173-176.

Hodson, P.V., B.R. Blunt, and D.J. Spry. 1978. Chronic toxicity of water-borne and dietary lead to rainbow trout (*Salmo gairdneri*) in Lake Ontario water. *Water Res.* 12:869-878.

Missouri Department of Natural Resources (MDNR). 1992. Mehan requests legal action against ISP for pollution of Big Creek. *MDNR News*:19(408).

Mushak, P., J.M. Davis, A.F. Crocetti, and L.D. Grant. 1989. Prenatal and postnatal effects of low-level lead exposure: integrated summary of a report to the U.S. Congress on childhood lead poisoning. *Environ. Res.* 50:11-36.

Schmitt, C.J., and W.G. Brumbaugh. 1990. National Contaminant Biomonitoring Program: Concentrations of arsenic, cadmium, copper, lead, mercury, selenium, and zinc in freshwater fishes of the United States, 1976-1984. *Arch. Environ. Contam. Toxicol.* 19:731-747.

Schmitt, C.J., F.J. Dwyer, and S.E. Finger. 1984. Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). *Can. J. Fish. Aquat. Sci.* 41:1030-1040.

Schmitt, C.J., M.L. Wildhaber, J.B. Hunn, T. Nash, M.L. Tieger, and B.L. Steadman. 1992. Biomonitoring of lead-contaminated Missouri streams with an assay for erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity in fish blood. U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Center, Columbia, MO. Project completion report to the U.S. Fish and Wildlife Service, Columbia, MO. 43 p.

Schmitt, C.J., M.L. Wildhaber, J.B. Hunn, T. Nash, M.L. Tieger, and B.L. Steadman. 1993. Biomonitoring of lead-contaminated Missouri streams with an assay for erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity in fish blood. *Arch. Environ. Contam. Toxicol.* 25:464-475.

U.S. Geological Survey. 1967. Mineral and water resources of Missouri. Vol. XLIII. U.S. Government Printing Office, Washington, D.C. 399 p.

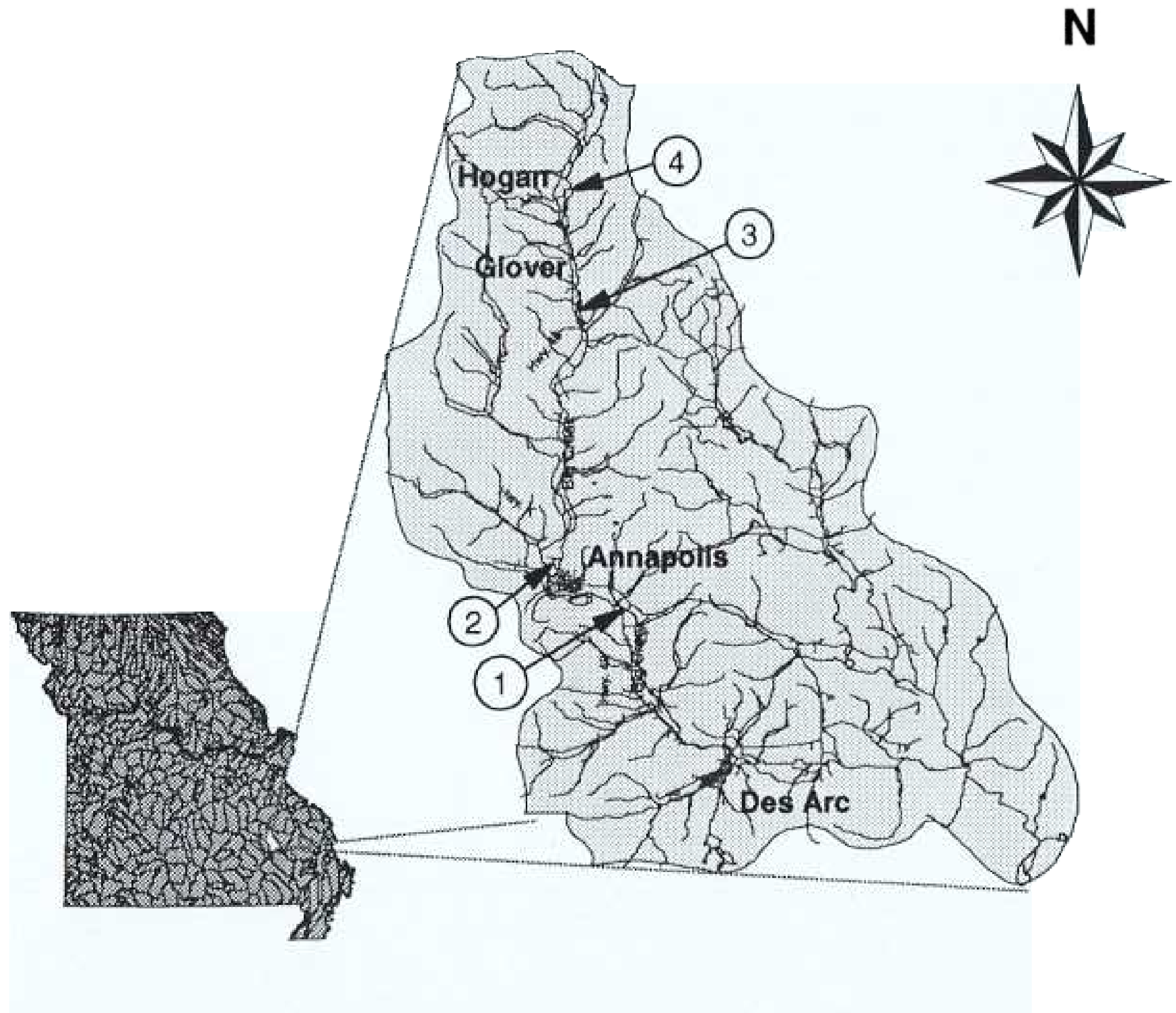
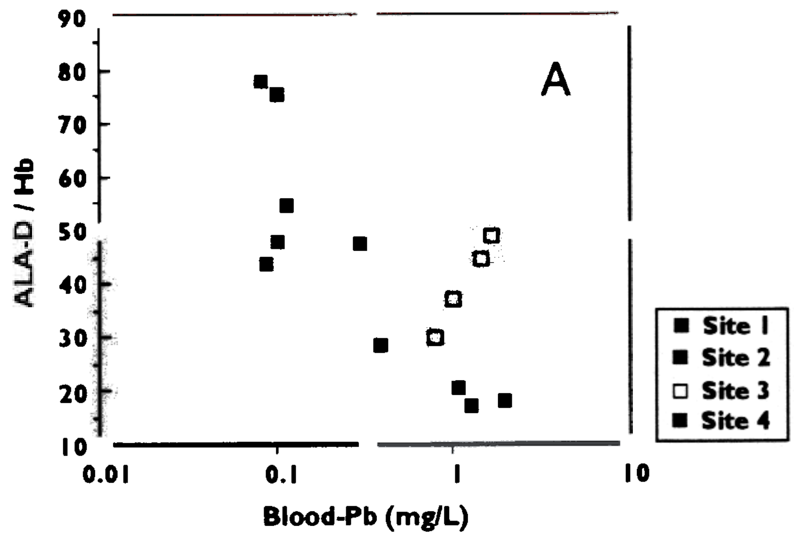


Figure 1. Big Creek collection sites, Iron Co., Missouri (see Table 1 for more ıformation)



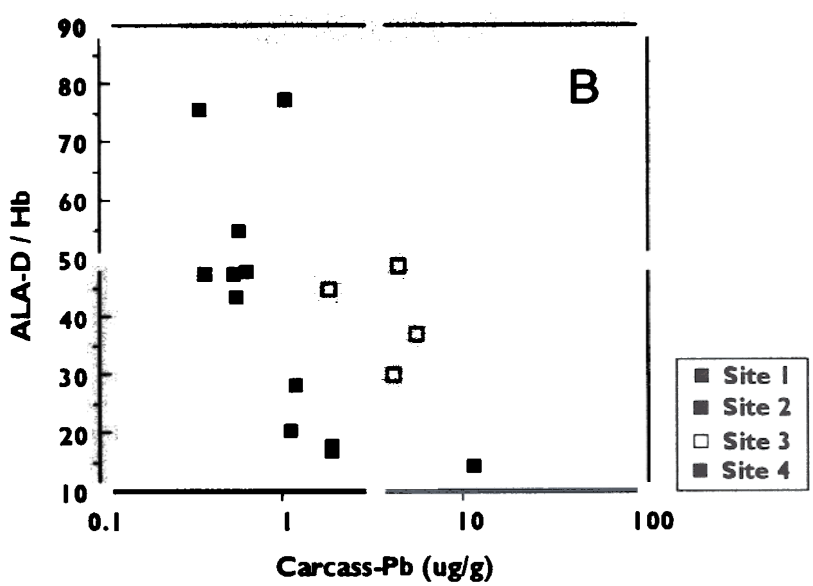


Figure 2. ALA-D / Hb vs. blood-Pb (A) and carcass-Pb (B)

Table 1. Big Creek collection sites (all in Iron Co., MO) and sample numbers

Site No.	Site Name	Site Location	Legal Description and (USGS 7.5-min quad.)	No. Fish Collected	No. ALA-D and Hb	No. Blood ICPES	No. Carcass ICPES
1	Big Creek below Annapolis	Pool above Hwy. 49 bridge	Union Twp., T31N-R3E, Sec. 24 (Des Arc)	8	8[a]	4	5
2	Big Creek above Annapolis	1st bridge above Rte. K (Br. 620)	Union Twp., T31N-R3E, Sec. 15 (Des Arc)	6	6	4	4
3	Big Creek at Glover	Weir below ASARCO smelter (Crane Lake)	Liberty Twp., T32N-R3E, Sec. 11 (Glover)	4	4	4	4
4	Big Creek at Hogan	Gravel lane NE of Hogan, along RR	Liberty Twp., T33N-R3E, Sec. 26 (Ironton)	4	4	3	4
$\sum$	--	--	--	22	22[a]	15	17

[a] for Hb, $n = 7$ and $\sum = 21$ at site 1

Table 2. Means of measurable carcass variables. Means containing the same subscript are not significantly different (*P* > 0.05, Fischer's protected LSD)

Site	Total Length[a]	Weight[b]	Al[c]	Cd[c]	Cu[c]	Mn[c]	Ni[c]	Pb[c]
1	335.6_a	434.5_a	8.1_a	1.71_a	1.01_a	13.4_a	0.19_a	2.21_a
2	229.8_b	109.0_b	66.9_b	1.82_a	1.00_a	12.7_a	0.17_a	0.70_b
3	256.0_b	161.5_b	5.7_a	1.32_a	0.83_a	15.9_a	0.20_a	3.66_a
4	204.3_b	78.3_b	86.9_b	0.22_b	1.02_a	20.4_a	0.33_b	0.46_b

[a] mm, arithmetic mean
[b] g, arithmetic mean
[c] μg/g (wet-weight), geometric mean

Table 3. Means of measurable blood variables. Means containing the same subscript are not significantly different ($P > 0.05$, Fischer's protected LSD)

Site	Hb[a]	ALA-D[b]	ALA-D / Hb	Al[c]	Be[c]	Cd[c]	Cu[c]	Mn[c]	Ni[c]	Pb[c]
1	7.72_a	227.8_a	28.5_a	0.26_a	$< 0.02_a$	0.44_a	0.77_a	0.24_a	0.03_a	1.04_a
2	7.14_a	440.7_b	57.9_{bc}	1.32_a	0.02_a	0.29_{ab}	0.86_a	0.27_a	0.06_a	0.11_b
3	7.84_a	318.5_a	40.3_{ac}	0.36_a	0.35_a	0.18_b	0.93_a	0.17_a	0.03_a	1.19_a
4	6.83_a	369.0_{ab}	54.8_b	0.28_a	0.28_a	0.03_c	1.09_a	0.17_a	0.11_a	0.13_b

[a] g/dL, arithmetic mean
[b] nmol PBG / g blood / h, arithmetic mean
[c] mg/L, geometric mean

n Big Creek, Iron Co., Missouri, August 30-31, 1993. See Table 1 for locations of collection sites (NA, not analyzed)

	Blood-Cu[b]	Blood-Mn[b]	Blood-Ni[b]	Blood-Pb[b]	Carcass-Al[c]	Carcass-Be[c]	Carcass-Cd[c]	Carcass-Cr[c]	Carcass-Cu[c]	Carcass-Mn[c]	Carcass-Ni[c]	Carcass-Pb[c]	Moisture (%)
	0.80	0.29	<0.02	2.00	5.89	<0.02	1.63	<0.06	1.40	12.83	0.18	1.90	69.0
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	0.73	0.21	0.07	1.30	4.35	<0.02	1.97	<0.06	0.81	12.94	0.13	1.88	68.9
	NA	NA	NA	NA	64.50	<0.01	1.09	<0.05	0.97	21.02	0.36	11.24	74.4
	0.65	0.21	<0.04	0.40	6.34	<0.02	1.99	<0.06	0.77	10.59	0.18	1.20	68.0
	0.95	0.25	0.05	1.11	3.42	<0.02	2.11	<0.06	1.28	11.88	0.22	1.09	68.9
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	1.00	0.84	0.05	0.09	54.96	0.014	1.42	<0.05	0.89	10.51	0.13	0.56	76.0
	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	0.71	0.27	0.20	0.13	77.95	<0.01	1.84	0.13	1.14	10.44	0.17	0.73	76.8
<0.10	1.30	0.20	0.04	0.11	85.92	<0.01	1.90	<0.05	1.17	24.76	0.27	1.03	73.4
<0.06	0.59	0.12	<0.06	<0.20	54.44	<0.01	2.22	<0.05	0.85	9.60	0.15	0.57	74.2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<0.03	0.91	0.13	0.03	1.73	10.39	<0.01	0.91	<0.05	0.67	14.94	0.23	4.43	74.1
<0.02	1.05	0.14	0.02	1.47	8.50	<0.01	1.66	<0.05	0.73	11.76	0.14	1.81	75.7
<0.20	1.10	0.20	0.06	0.80	12.42	<0.01	1.71	<0.05	1.12	16.41	0.24	4.11	74.6
<0.30	0.72	0.25	<0.05	1.00	0.96	<0.01	1.18	<0.05	0.88	22.03	0.23	5.44	73.2
<0.04	1.20	0.13	0.06	0.09	165.91	<0.02	0.38	0.24	1.27	18.14	0.38	0.63	76.5
NA	NA	NA	NA	NA	63.74	<0.01	0.18	<0.05	0.95	25.60	0.36	0.38	74.4
0.100	1.10	0.15	0.07	0.09	51.91	<0.01	0.15	0.10	0.91	17.17	0.26	0.35	74.3
0.700	0.99	0.26	0.30	0.28	104.00	<0.01	0.25	0.23	1.03	21.58	0.33	0.54	75.0